

CLAIMS

1. A method for preventing proliferation of a tumor, comprising:
exposing a tumor cell to an effective amount of heparinase III for preventing
proliferation of the tumor cells in order to prevent growth of the tumor.
5
2. The method of claim 1, wherein the heparinase III is a modified heparinase
III.
3. The method of claim 1, wherein the heparinase III is a native heparinase III.
10
4. The method of claim 1, wherein the heparinase III is injected into the tumor
in vivo.
5. The method of claim 1, wherein the heparinase III is administered
15 systemically to a subject having a tumor.
6. The method of claim 1, wherein the heparinase III is administered orally to a
subject having a tumor.
- 20 7. The method of claim 1, wherein the tumor cell is administered the heparinase
III *in vitro*.
8. The method of claim 1, wherein the heparinase III is administered in
conjunction with an anti-cancer drug.
25
9. The method of claim 1, wherein the heparinase III is administered to a subject
having a non-metastatic tumor in order to prevent the tumor from becoming metastatic.
10. The method of claim 1, wherein the tumor is selected from the group
30 consisting of a prostate tumor and melanoma.

11. The method of claim 1, wherein the heparinase III is administered to a subject without any additional anti-cancer drugs.

12. A method for preventing tumor cell metastasis, comprising:
5 exposing a tumor cell to an effective amount of heparinase III for preventing invasion of the tumor cell across a barrier.

13. The method of claim 12, wherein the heparinase III is a modified heparinase
10 III.

14. The method of claim 12, wherein the heparinase III is a native heparinase III.

15. The method of claim 12, wherein the heparinase III is administered *in vivo* in
15 conjunction with an anti-cancer drug.

16. The method of claim 12, wherein the barrier is an *in vivo* cell barrier.

17. The method of claim 12, wherein the barrier is an *in vitro* barrier of an
20 extracellular matrix coated membrane.

18. A method for preparing therapeutic agents for the treatment for a tumor,
comprising:
isolating at least a portion of a tumor,
treating the portion of the tumor with heparinase III to produce HLGAG
25 fragments, and
isolating the HLGAG fragments, wherein the HLGAG fragment is the therapeutic
agent.

19. The method of claim 18, further comprising determining the sequence of the
30 HLGAG fragments.

20. A method for treating a subject having a tumor, comprising, administering to the subject a therapeutic HLGAG fragment to treat the tumor.

21. The method of 20, wherein the therapeutic HLGAG fragment administered
5 to the subject is a synthetic HLGAG fragment generated based on the sequence of the HLGAG fragment identified when the tumor is contacted with heparinase III.

22. The method of 20, wherein the therapeutic HLGAG fragment administered
to the subject is an isolated HLGAG fragment produced when the tumor is contacted
10 with heparinase III.

23. A composition, comprising:
heparinase III or a therapeutic HLGAG fragment in an effective amount for
preventing metastasis of a tumor cell and a targeting molecule for targeting the
15 heparinase III to the tumor, in a pharmaceutically acceptable carrier.

24. The composition of claim 23, wherein the heparinase III is a modified
heparinase III.

20 25. The composition of claim 23, wherein the heparinase III is a native
heparinase III.

26. The composition of claim 23, wherein the targeting molecule is a compound
which binds specifically to an antigen on the surface of a tumor cell.

25

27. A composition, comprising:
heparinase III or a therapeutic HLGAG fragment in an effective amount for
preventing metastasis of a tumor cell and an anti-cancer compound in a pharmaceutically
acceptable carrier.

30

28. A substantially pure heparinase III, comprising:

a polypeptide having the amino acid sequence of the mature peptide of SEQ ID NO: 2 or having conservative substitutions thereof within residues non-essential to enzymatic function, wherein at least one histidine residue selected from the group consisting of His 36, His105, His110, His139, His152, His225, His234, His241, His424, His469, and
5 His539 has been substituted with a residue selected from the group consisting of alanine, serine, tyrosine, threonine, and lysine.

29. The substantially pure heparinase III of claim 28, wherein the polypeptide has at least one substitution within a histidine residue selected from the group consisting
10 of His110 and His241.

30. The substantially pure heparinase III of claim 28, wherein the polypeptide has a substitution at His110.

15 31. The substantially pure heparinase III of claim 29, wherein the polypeptide has a substitution at His241.

32. A substantially pure heparinase III comprising:
a modified heparinase III having a modified product profile, wherein the modified
20 product profile of the modified heparinase III is at least 10% different than a native product profile of a native heparinase III.

33. A substantially pure heparinase III comprising:
a modified heparinase III that can cleave a heparan sulfate substrate having a
25 modified heparinase III k_{cat} value, wherein the modified heparinase III k_{cat} value is at least 10% different than a native heparinase III k_{cat} value.

34. A pharmaceutical preparation comprising a sterile formulation of the substantially pure heparinase III of any one of claims 28-33 and a pharmaceutically
30 acceptable carrier.

35. An immobilized substantially pure modified heparinase III comprising:

a modified heparinase III as in any one of claims 28-33, and
a solid support, wherein the modified heparinase III is immobilized on the solid support.

36. A method of specifically cleaving a heparin-like glycosaminoglycan,
5 comprising:
contacting a heparin-like glycosaminoglycan with the modified heparinase III of any
one of claims 28, 32, or 33.

37. The method of claim 36, wherein the method is a method of removing active
10 HLGAG fragments from a HLGAG fragment containing fluid.

38. The method of claim 36, wherein the heparinase III is immobilized on a solid
support.

15 39. The method of claim 36, wherein the method is a method for inhibiting
angiogenesis and wherein an effective amount for inhibiting angiogenesis of the
heparinase III is administered to a subject in need of treatment thereof.

40. The method of claim 36, wherein the heparinase III is administered to a
20 tumor.

41. The method of claim 36, wherein the heparinase III is administered in a
biodegradable, biocompatible polymeric delivery device.

25 42. The method of claim 36, wherein the heparinase III is administered in a
pharmaceutically acceptable vehicle for injection.

43. The method of claim 42, wherein the heparinase III is administered in an
effective amount for diminishing the number of blood vessels growing into a tumor.

30 44. The method of claim 36, wherein the heparinase III is administered in a
pharmaceutically acceptable vehicle for topical application to the eye.

45. The method of claim 44, wherein the heparinase III is administered in an effective amount for diminishing the symptoms of an eye disease characterized by abnormal neovascularization.

5

46. The method of claim 36, wherein the heparinase III is administered in a pharmaceutical vehicle suitable for topical application.

47. The method of claim 36, wherein the heparinase III is administered in an effective amount for diminishing the symptoms of psoriasis.

10

48. The method of claim 36, wherein the method is a method for inhibiting cellular proliferation.

49. The method of claim 36, wherein the method is a method for sequencing HLGAG fragments.

15

50. A method comprising treating or preventing a subject having a cancer or at risk of developing a cancer by administering to the subject a therapeutic HLGAG fragment.

20

51. The method of claim 50, wherein the therapeutic HLGAG fragment is a composition of HLGAG fragments wherein at least 50% of the HLGAG fragments are di- or tri- sulfated disaccharides.

52. The method of claim 50, wherein the therapeutic HLGAG fragment is a composition of HLGAG fragments wherein at least 75% of the HLGAG fragments are di- or tri- sulfated disaccharides.

25

53. The method of claim 50, wherein the therapeutic HLGAG fragment is a composition of HLGAG fragments wherein at least 90% of the HLGAG fragments are di- or tri- sulfated disaccharides.

30

54. The method of claim 50, wherein the therapeutic HLGAG fragment is free of mono- or un- sulfated disaccharides.

55. A method for preparing LMWH, comprising:
5 contacting an HLGAG sample with a modified heparinase III molecule to produce LMWH.

56. A composition, comprising, the LMWH produced by the method of claim 55.

10 57. A method for treating or preventing a disorder associated with coagulation, comprising:
 administering to a subject an effective amount of the composition of claim 56 to treat or prevent a disorder associated with coagulation.

15 58. A method for treating or preventing a tumor, comprising:
 administering to a subject an effective amount of the composition of claim 56 to treat or prevent a tumor in the subject.

59. A method for treating or preventing psoriasis, comprising:
20 administering to a subject an effective amount of the composition of claim 56 to treat or prevent psoriasis in the subject.

60. A method for treating or preventing neovascularization, comprising:
 administering to a subject an effective amount of the composition of claim 56 to
25 treat or prevent neovascularization in the subject.